Flirtations with Phage Therapy

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Lytic bacteriophages (phages for short) are viruses that can infect and kill bacteria. Phages are diverse, numerous and ubiquitous. Most species of bacteria that have been studied in sufficient depth have been found to be susceptible to infection by at least one kind of phage. In general, phages are very host-specific. Indeed, some bacterial species are categorised into subgroups on the basis of their phage susceptibility. Conversely, a particular bacterial strain may be susceptible to infection by many different phage types. Phages have been studied intensively since the 1940s and have contributed enormously to our understanding of microbial genetics and molecular biology. Today, phages are routinely used as 'tools' in many molecular biological laboratories. The discipline of virology has its roots in the study of bacteriophages.

It has been roughly estimated that, worldwide, there are about ten times as many phage particles as there are bacteria. By extrapolation, this means that each of us has many more phage particles in our bodies (infecting our gastrointestinal flora) than we have mammalian cells.

The idea of using phages to combat bacterial infections is far from new. Phages were discovered independently by Frederick Twort* and Félix d'Herelle in 1915 and 1917, respectively. The discovery of phages, in the pre-antibiotic era, engendered great optimism among scientists and physicians worldwide. D'Herelle—who worked at the Pasteur Institute in Paris—rapidly and vigorously began investigating phage therapy. He first carried out animal experiments, investigating whether or not phage therapy was beneficial to chickens with salmonellosis. He also studied rabbits with *Shigella dysenteriae* infection and water buffaloes with pasteurellosis. Having satisfied himself that phage therapy looked promising, he proposed to conduct human therapeutic trials. Before doing so, he swallowed and subsequently injected himself and members of his family with increasing amounts of phage to satisfy himself that phage administration would be safe. Co-workers were also dosed. His first report on phage therapy in a human subject was published in 1921 and described startling success in the treatment of a near-fatal case of bacillary dysentery. His subsequent report of apparently successful treatment of bubonic plague (by direct injection of phages into 'buboes' – infected lymph nodes) grabbed considerable attention.

While phage therapy (as practised in the first half of last century) seemed to be very safe, it was not always effective. The many reasons for this are beautifully reviewed by Summers

* Perhaps of interest to British veterinarians, Twort, co-discoverer of bacteriophages, worked for part of his career as the Superintendent of the Brown Institute in London. This institute, founded in 1871 using the legacy of a wealthy lawyer, was dedicated to looking after sick animals at little or no cost to the owners. It was essentially the country's first animal hospital and veterinary pathology laboratory. It conducted valuable research into animal diseases including Rinderpest and Johne's disease. The Brown Institute was destroyed in 1944, when it was hit by German flying bombs. Some of the money left over from the sale of the land upon which it had stood was used to endow the Thomas Brown research fellowship in veterinary pathology at the Royal Veterinary College. For more information see: http://www.vauxhallsociety.org.uk/Brown.html

(2001). Incomplete understanding of phage biology posed major problems. For example, it was not even appreciated that phages were viruses until long after they began to be used in therapeutic trials; some researchers thought they might be enzymes. Some commercial 'phage medications' actually contained preservatives that completely inactivated the phages they purported to contain.

Then, in the 1940s, the startling efficacy of the 'new' antibiotics eclipsed phage therapy and led to a substantial decline of interest in western countries, particularly the United States. Phage therapy continued to be used in Germany, Russia and the rest of Eastern Europe, where potent antibiotics were not always readily available when needed. Interestingly, some medical packs seized from Rommel's soldiers during the Second World War were found to contain phage remedies (Summers, 2001). In parts of Eastern Europe, the commitment to development and use of phage therapy remained remarkably strong. For example, shortly after World War II, 1300 people were employed by the Eliava Institute in Tbilisi, Georgia. This facility was founded by d'Herelle and Georgyi Eliava and, to this day, remains actively involved in the development and utilisation of phage therapy.

Throughout the Cold War, numerous publications (mostly written in Russian or other East European languages) described the successful use of phages for treatment of a variety of bacterial infections. Unfortunately, many of these reports described clinical experiences and 'experiments' that were not rigorously controlled. At the time these studies were carried out, understanding of phage biology was still quite limited. These scientific limitations and the fact that much of the work was being carried out in the USSR gave phage therapy a so-called "Soviet taint" (Summers, 2001). The overall effect was to produce considerable mistrust, deep scepticism and abandonment of phage therapy in the West.

In the late 1960s, a World Health Organisation-sponsored investigation of phage therapy for human cholera was carried out in East Pakistan. Two studies were carried out sequentially and the results were reported separately. In the first report, it was noted that a high dose of anti-cholera phage was equivalent to tetracycline in reducing the excretion of vibrio organisms in faeces, but that this did not translate into a convincing improvement in clinical outcome (i.e. shorter hospital stay, more rapid resolution of diarrhoea). In a second, much larger study, patients were randomised and placebo-administered control patients were included. Orally administered phage was compared with oral and intramuscularly administered phage and tetracycline. All patients received fluid therapy, as needed. This second, larger study provided a real opportunity to get to grips with whether or not phage therapy compared favourably with conventional therapy for treating cholera and reducing environmental bacterial contamination. Unfortunately, the amount of phage administered in the second study was at least three orders of magnitude lower than the dose used in the first study and, in absolute terms, was very low indeed. Phage therapy was not shown to be beneficial in the second study.

In the 1980s, some excellent research done in the UK by Williams Smith and his colleagues addressed many of the weaknesses of earlier studies (Smith and Huggins 1982, 1983; Smith et al 1987a, 1987b). In a series of landmark studies, these researchers investigated the use of phage therapy in farm animals, including calves, piglets and lambs. They also carried out rigorous experimental studies in mice. They focussed upon treatment of diarrhoea caused by a particular, well characterised strain of *E. coli.* In 1987, they showed that severe, experimental diarrhoea in calves could be cured by a single dose of 100,000 phage particles and could be prevented by doses as low as 100 particles. They obtained their phages from ordinary sewage.

More recently, similar work has been done on *E. coli*- and *Campylobacter*-infected chickens, with promising results.

There has been some interesting research investigating the approach of administering bacteriophages to farm animals in the immediate pre-slaughter period, with the intention of reducing contamination in the abattoir by particular bacterial pathogens.

Studies have shown that some bacterial diseases of fish may be effectively controlled using phage therapy. These studies show particular promise because intensively farmed fish live in a suspension of bacteria and bacteriophages, conditions that are very similar to those used in laboratories to grow bacteriophages.

At Massey University, we have begun to investigate the potential use of phage therapy in dogs and cats.[†] We chose to focus, initially, upon uropathogenic strains of *E. coli*. Our first manuscript on this subject is in preparation. Like Williams Smith and his colleagues, we had no difficulty in finding—in ordinary city sewage—abundant, diverse phages able to kill canine and feline uropathogenic *E. coli* isolates. The phages in our collection vary widely in their killing ability. Some are able to kill only one or two strains of uropathogenic *E. coli*, whereas others have much broader killing ability. We have checked for the presence or absence of a selection of about 30 virulence gene markers in each member of our collection of uropathogenic *E. coli* isolates (Freitag et al 2005). Some of the *E. coli* virulence genes we have studied encode cell surface molecules that phages may use to enter their bacterial hosts. We are investigating whether or not positive results for particular virulence gene markers correlate with susceptibility to killing by phage. If we find any such correlations, it is conceivable that some of our phages may not only be able to kill their target bacteria, but may also promote the emergence of phage-resistant mutants that lack certain urovirulence traits and are, therefore, less pathogenic and perhaps more readily cleared from the body. This is similar to what was observed by Williams Smith and his co-workers in 1987. Their phages targeted only K1-positive *E. coli* and, when phage resistant clones of *E. coli* emerged, they were found to be K1-negative and less pathogenic than the parent strain.

Substantial hurdles will need to be overcome if phage therapy is ever to become a part of routine clinical practice. For example, it is possible that the complexities of host-phage interactions *in vivo*, and the potential advantages of sometimes using mixtures of phages (so-called 'phage cocktails') rather than single phage clones, will make it difficult or impossible for live phage preparations ever to be approved by regulatory authorities and commercialised in a conventional way by pharmaceutical companies. Phage components, which can be standardised much more readily, have a far greater chance of being commercialised in the near future. For example, some phage tail preparations can attach to bacterial cells and punch holes in their cell walls leading to bacterial cell death. However, use of standardised phage-derived enzymes, or other phage components, seems relatively mundane and rather like conventional antibiotic therapy. Products of that kind would not take advantage of the highly enticing 'self-replicating' capability of phages. They would not exploit the amazing 'generosity of nature' in providing such an enormous diversity of host-specific phages. Nor would they capitalise upon the ability of phages to co-evolve with their bacterial hosts.

Despite these and many other significant challenges, there are reasons to suppose that phage therapy may, in future, play a role in canine and feline medicine. Some recently published, high quality research articles are encouraging and make this a very enjoyable field of study.

Further reading

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